Differential Germination of Fern and Moss Spores in Response to Mercuric Chloride

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The pervasive use of toxic substances and the synthesis of myriad new such compounds requires that toxic substance limits be set and that environmental monitoring be maintained in order to insure the health and integrity of the biosphere. Bioassays are the primary mechanism for meeting these two requirements. A bioassay designed to determine the environmental impact of a particular toxic substance, for example divalent mercury, should have the following attributes: ease of performance, low cost, brevity, sensitivity, and high overall reflectivity of environmental stress induced by the presence of the toxic substance. The appropriateness of a bioassay is based on the selection of its two primary components—the living system employed and the parameter measured.

Petersen et al. (1980) have demonstrated the feasibility of using the germination of *Onoclea sensibilis* spores to gauge the toxicity of various heavy metal ions. It was found that for the three metal ions tested, toxicity was directly proportional to atomic weight. Hg^{++} is twice as toxic as Cd^{++} and four times as toxic as Co^{++}. The present study is a comparison of the germination responses of different fern and moss spores to divalent mercury.

There are few investigations on the effects of metal ions and other potential pollutants on fern spores and gametophytes that yield data pertinent to pollution research and control. Nakazawa and Tsusaki (1959) determined that the fern spore cytoplasm associated with rhizoid differentiation has a marked affinity for metal ions. Nakazawa and Otaki (1962) demonstrated the affinity of developed rhizoids for metal ions. Fern rhizoids apparently function like the root hairs of vascular plants in absorbing water and minerals from the soil. Therefore, their affinity for metal ions is not surprising. Co^{++} and Ni^{++} were shown to prolong filamentous (one-dimensional) growth in *Lygodium smithianum* gametophytes (Parés, 1958) resulting in a retardation of their development sequence. LiCl caused a precocious differentiation of terminal papillae (gland-like hair cells produced by some fern gametophytes) in *Dryopteris varia* (Nakazawa, 1960a, b). Several metal chlorides at concentrations of 0.005–0.08M decreased the period of fern sperm motility (Igura, 1958).

A few papers on ferns with direct application to pollution monitoring have been published. Klekowski (1976), Klekowski and Berger (1976), and Klekowski and Poppel (1976) found that meiotic chromosome behavior during fern sporogenesis was correlated with the presence of toxic substances in the environment. Howard and Haigh (1972) studied the effects of increasing doses of X-radiation on the first mitotic division of *Osmunda regalis* spores. Edwards and Miller (1970, 1972a, b) studied the quantitative effects of ethylene on *Onoclea sensibilis* spore germination and gametophyte growth. Fern gametophytes also have been successfully employed in bioassay procedures for the plant hormones kinetin, gibberelic acid, and antheridogen (Bopp, 1968; Brandes, 1973).

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Mosses are especially adapted to absorb and concentrate substances present in the atmosphere, and so moss gametophytes have proven to be sensitive indicators of airborne pollutants (Huckabee, 1973; Skaar et al., 1973; Little & Martin, 1974). Francis and Petersen (1980) reported on the synergistic effects that metal ion combinations had on the germination of *Polytrichum commune* spores.

Spores of *Onoclea sensibilis*, *Osmunda cinnamomea*, and *Osmunda claytoniana* and the moss *Polytrichum commune* were selected for investigation because they are of wide distribution and frequent occurrence and all produce copious quantities of easily collected spores. In addition, *O. sensibilis* had been employed in the development of the fern spore heavy metal bioassay (Petersen, et al., 1980). The two *Osmunda* species represented a fern family distinct from that of *Onoclea* and would yield data on intrageneric differences in response to heavy metal ions. *Polytrichum commune* was selected to compare its heavy metal response with those of the ferns.

TABLE 1. SPORE GERMINATION IN VARIOUS Hg^{++} CONCENTRATIONS EXPRESSED AS A PERCENTAGE OF CONTROL GERMINATION.[1]

Hg^{++} ppm	*O. sensibilis*	*O. cinnamomea*	*O. claytoniana*	*P. commune*
0.0	100	100	100	100
0.2	101	98	96	77
0.4	98	91	89	30
0.6	98	77	33	8
0.8	88	60	20	0
1.0	85	13	14	0
1.5	75	8	-	0
2.0	68	1	1	-
3.0	55	0	0	-
4.0	30	-	-	-
6.0	9	-	-	-
8.0	1	-	-	-
10.0	0	-	-	-
20.0	0	-	-	-

[1]Tests were not run, or the experiment was lost, where no numerical value is given.

MATERIAL AND METHODS

Approximately 10,000 spores of each species were cultured in a petri dish 60mm diam. in 8 ml of full strength liquid Knudson's medium at a pH 5.5 with 0–20 ppm of divalent mercury ion (Hg^{++}) added as $HgCl_2$. The dishes were sealed in clear plastic sandwich bags and cultured in a Sherer Growth Chamber at 20°C in 300 ft-c of continuous light from cool-white fluorescent lamps. Spore germination was considered to have occurred when the spores produced a rhizoid or the first prothallial cell divided. After eight days, 500 spores were examined per plate. Three replicates were run for each species tested at each Hg^{++} concentration.

RESULTS AND DISCUSSION

The germination of the controls (0 ppm HG^{++}) was uniformly high; *O. sensibilis* had 88%, *O. cinnamomea* 92%, *O. claytoniana* 96%, and *P. commune* 94%. Spore germination at each Hg^{++} concentration except one was lower than in the controls. Germination was expressed as a percentage of each species' control germination (*Table 1*). These results are summarized in terms of the standard bioassay toxicity values of LC_{50} and LC_{100}, which are presented both in terms of ppm and μM (*Table 2*). LC_{50} is the concentration of a toxic substance necessary to kill 50% of the organisms from a control value, and LC_{100} is the minimum concentration necessary to kill 100%.

TABLE 2. Hg^{++} LC_{50} AND LC_{100} VALUES FOR FOUR TAXA.

Toxicity value	*O. sensibilis*	*O. cinnamomea*	*O. claytoniana*	*P. commune*
LC_{50}				
ppm	3.2	0.82	0.53	0.30
μM	16	4.1	2.6	1.5
LC_{100}				
ppm	10	3.0	3.0	0.8
μM	50	15	15	4.0

A comparison of spore germination responses shows that *P. commune* is the taxon most susceptible to HG^{++} and is ten times more sensitive than *O. sensibilis*, which is the least susceptible. The two *Osmunda* species have intermediate and similar HG^{++} toxicity values. They are approximately four times more sensitive to HG^{++} than is *O. sensibilis* (*Table 2*). Therefore, based on the sensitivity of response, *P. commune* would be the species of choice in a mercury ion bioassay.

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